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Research Article

Two alien species of Branchiobdellida (Annelida: Clitellata) new to the British Isles: a morphological and molecular study

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Abstract

Freshwater ecosystems are particularly vulnerable to the effects of alien species and decapod crustaceans, notably crayfish, are a principal threat. Although symbiotic fauna may influence the impact and dispersal of introduced species, this is often overlooked. Here we provide the first record of non-native ecto-symbiotic branchiobdellidan worms on invasive signal crayfish (*Pacifastacus leniusculus* Dana, 1852) in the British Isles. Using morphological and molecular techniques we identified and re-described two branchiobdellidan species new to the UK, *Xironogiton victoriensis* Gelder and Hall, 1990 and *Cambarincola* aff. *okadai* Yamaguchi, 1933, both of which were found at a single location in the Gavenny River, South Wales. The prevalence of *X. victoriensis* and *C.* aff. *okadai* was 75.34% and 71.23% respectively. Although the level of *X. victoriensis* and *C.* aff. *okadai* co-infection was high at 75.41% of all infected animals, the two species exhibited different micro-habitat preferences on the host with the former being found predominantly on the chelae and walking legs and the latter on the carapace and abdomen. For both branchiobdellidan species, worm burdens were positively correlated with crayfish size. The lack of branchiobdellidan records from signal crayfish in nearby water bodies, and the reports of native white clawed crayfish (*Austropotamobius pallipes*) in the Gavenny as recently as 2000, indicates that introduction of this worm infested population occurred relatively recently, despite stringent legislation banning the import and transportation of non-native crayfish into the UK.

Key words: invasive species, *Cambarincola okadai*, *Xironogiton victoriensis*, signal crayfish, *Pacifastacus leniusculus*

Introduction

Invasive species are a principal threat to global biodiversity (Wilcove et al. 1998), but it is often overlooked that their dispersal and impact on native biota may be influenced by symbionts (Torchin et al. 2002). North American crayfish species are among the most successful and widespread invasive species whose effects on native crayfish are exacerbated when infected with *Aphanomyces astaci* (Schikora, 1906), the causative agent of crayfish plague (Unestam and Weiss 1970; Holdich and Reeve 1991; Kozubíková et al. 2009; Schrimpf et al. 2013). Whilst North American crayfish are largely resistant to this parasite, they act as reservoirs and vectors of the disease, increasing its transmission to susceptible native European crayfish in which infection is reportedly always lethal (Unestam and Weiss 1970). Whilst the majority of studies on crayfish symbionts are focused on *A. astaci*, crayfish are host to several other fungi, viruses, bacteria, protists, helminths and annelids (reviewed by Longshaw 2011). One group of organisms that are frequently introduced on invasive crayfish are branchiobdellidan worms (Gelder 1996). These ectosymbiotic annelids live primarily on astacoidean crayfish (Govedich et al. 2009) and are considered obligate ectosymbionts, as reportedly their cocoons only embryonate if attached to a live host (Govedich et al. 2009).

The relationship between crayfish and branchiobdellidans can vary across the symbiosis continuum from mutualism (e.g. Brown et al. 2002, 2012; Lee et al. 2009) to commensalism (e.g. Bishop 1968; Keller 1992; Govedich et al.

Table 1. Total number, number of crayfish per hour (catch-per-unit-effort, CPUE) and species of crayfish (either invasive *Pacifastacus leniusculus* or native *Austropotamobius pallipes*) found at each of the sites (approximate National Grid References, NGRs, included) manually surveyed during July-Oct 2012.

Site name	NGR	No. crayfish	CPUE	Species
Nant Glandulas	ST194839	1	0.25	*P. leniusculus*
Bachowey 1	SO158464	2	0.25	*P. leniusculus*
Bachowey 2	SO168457	37	4.44	*P. leniusculus*
Sirhowey 1	ST178961	19	3.38	*P. leniusculus*
Gavenny	SO308164	27	3.95	*P. leniusculus*
Mochdre	SO086904	32	5.13	*P. leniusculus*
Lugg at Humber	SO522523	23	3.45	*P. leniusculus*
Back Brook	SO302569	11	1.78	*A. pallipes*
Dulas Brook	SO353321	1	0.2	*A. pallipes*
Gurrey Fach	SN622252	0	N/A	N/A
Sirhowey 2	ST184995	0	N/A	N/A
Dowlais Brook	ST309927	0	N/A	N/A
Knobley Brook	SO279607	0	N/A	N/A
Curl Brook	SO333570	0	N/A	N/A
Dore at Peterchurch	SO344385	0	N/A	N/A

2009) and parasitism (Vogt 1999; Brown et al. 2012; Rosewarne et al. 2012) depending on host, branchiobdellidan species and density, and environmental conditions. Branchiobdellidans, therefore, have the potential to affect the invasion success of crayfish either facilitatively or detrimentally. Despite this branchiobdellidans are relatively understudied and their distribution on invasive crayfish in the British Isles has not been assessed. To our knowledge there are only three previous reports of branchiobdellidans (*Branchiobdella astaci* Odier, 1823) in the UK (Leeke and Price 1965; Rogers et al. 2003; Rosewarne et al. 2012), all on native white clawed crayfish (*Austropotamobius pallipes* Lereboullet, 1858).

Here we surveyed 17 sites for signal crayfish (*Pacifastacus leniusculus*) in Wales and bordering parts of England (Herefordshire) of which one contained crayfish infected with branchiobdellidans. Using a combination of morphological and molecular techniques we identified two species of branchiobdellidans in Wales, *Xironogiton victoriensis* (Gelder and Hall, 1990) and an unknown species putatively identified as *Cambarincola* aff. *okadai* that is morphologically similar to, but genetically distinct from, *C. okadai* (Yamaguchi 1933). This is the first know record of branchiobdellidans on invasive crayfish in the UK. From field survey data we examined the prevalence, mean intensity and micro-habitat use of these branchiobdellidans on the crayfish host. We present detailed morphological descriptions of both species that can be compared against alien branchiobdellidans subsequently found in the UK or mainland Europe to help monitor non-native species' movements. Our species descriptions will also assist in the future identification of *C.* aff. *okadai.*

Methods

Field surveys

The UK national crayfish database, CrayBase (James et al. 2014), was used to identify sites positive for signal crayfish populations in Wales and bordering Herefordshire (England) between 1975 and 2012. Each of the 17 sites was surveyed once during July-October 2012 (Table 1), using either standard stone turning and kick sampling protocols (n=15 sites) or baited traps (n = 2 sites) left to fish overnight, where manual surveying was unsuitable. Upon capture, the external surfaces of all crayfish were examined for branchiobellidans. All crayfish were sexed, measured (carapace length; mm) and any signs of disease recorded. Additionally we noted if crayfish were in inter-moult (carapace hard), pre/post-moult (carapace hard but could be depressed) or moult (carapace completely soft) condition. As pre and post-moult crayfish are difficult to definitively discriminate these categories were combined.

At the site where branchiobdellidans were located in 2012 an additional six manual surveys were conducted (one in Oct 2012, and five between April and June 2013) to determine the prevalence, mean intensity and distribution of worms on the

crayfish. A sub-sample of worms (n = 30, Oct 2012 collection) were carefully removed from the external surface of the crayfish (n = 2) using forceps and preserved in 90% molecular grade ethanol for subsequent identification. Analysis of these branchiobdellidans indicated the presence of at least two branchiobdellidan species. Therefore for subsequent surveys conducted in 2013, all worms were removed from the external surface of the crayfish host (their position noted) and stored separately in 90% ethanol according to the location on the crayfish from which they were removed. Furthermore, a subset of crayfish (n = 10) was dissected to examine the gill tissue for branchiobdellidans.

Morphological identification

Branchiobdellidans were examined live in the laboratory, narcotized by the gradual addition of 7% $MgCl_2$ (later fixed in 4% formalin), or preserved in 100% ethanol. Some specimens were stained temporarily with alcohol solutions of Methyl Green (e.g., Mackie and Gobin 1993) or Shirlastain A (Petersen 1998) to aid observation of the morphological characteristics. Internal features were examined following dissection.

Photographs of live worms were taken using Live View image capture in Manual mode with mirror lock-up enabled (Canon EOS Utility 2.10.4; Apple Intel Core 2 Duo MacBook Pro) via a stand-mounted Canon EOS 550D camera, fitted with an EFS 60mm or MP-E 65mm (1-5x) macro lens and a cable mounted Speedlight 580EX II Flash. Raw images were edited in Apple Aperture 3.5.1 and all final figures prepared in Adobe Photoshop Elements 12.0. Drawings and measurements were made using a camera lucida attachment on a Wild M8 stereo-zoom or Nikon Labophot-2 compound microscope. Morphometric analyses were made using Statview 4.5 on an Apple G4 Powerbook laptop in Classic mode. Cited material is deposited in the National Museum Wales, Cardiff (NMW).

Molecular identification

Using the HotSHot protocol, genomic DNA was individually extracted from eight branchiobdellidans (four of each species) in 99 µl TE buffer including 5 µl of proteinase K and 0.45% Tween 20 incubated at 56ºC for 4 h and neutralised at 95°C for 10 min (Truett et al. 2000). Universal mitochondrial cytochrome *c* oxidase I (COI) primers modified from Folmer et al. (1994), forward: 5' GGT CAA CAA ATC ATA AAG AYA TYG G 3', and reverse: 5' TAA ACT TCA GGG TGA CCA AAR AAY CA 3' or 5'-AAGAGCGACGG GCGATGTGT-3' (Harper et al. 2005) were used to amplify a ~700bp fragment. Higher quality sequences were obtained using the latter reverse primer. PCR consisted of 1 µl DNA template, 1.5 µl buffer, 1.5 µl magnesium chloride (25mM), 0.3 µl ddNTP mix (10 mM), 1 µl of each primer (10 µM), 0.2 µl FermantasTaq DNA polymerase (5U/µl), and water to a total volume of 15 µl. A negative control (with no DNA template) was included with each reaction. PCR conditions were as follows: initial 180s denaturation at 95°C; 35 cycles of 30s at 94°C, followed by 40s at 48°C and 70s at 72°C; final 12 min elongation at 72°C. PCR products were purified using Exonuclease I and Shrimp Alkaline Phosphatase and sequenced using the same primers at Cardiff University Molecular Biology Support Unit. Forward and reverse amplicons were aligned and edited using Sequencher™ version 4.7 and subsequently compared against all branchiobdellidan sequences available on Genbank.

Statistical analysis

Separate negative binomial generalized linear mixed models (GLMMs) were run to investigate factors influencing the infection intensity of each branchiobdellidan species on signal crayfish. Crayfish moult stage (inter-moult, pre and post-moult or in moult), size (carapace length, mm) sex/life stage (juvenile, male or female) and an interaction between the latter two variables, were included in both models. Additionally, chelae number (0, 1 or 2) was included as a random effect in these GLMMs. Models were refined by stepwise deletions of non-significant terms from the starting model using Analysis of Variance (Crawley 2007). Model fit was assessed through visual examination of Pearson's residual plots, as according to Thomas et al. (2013). All data analyses were conducted using the glmmADMB package in R statistical software version 3.02.

Results

Field survey

Of the 17 sites surveyed for crayfish in Wales during July-October 2012, invasive signal crayfish (*Pacifastacus leniusculus*) were found at nine and native white clawed crayfish (*Austropotamobius pallipes*) at two locations (Table 1; data added to CrayBase, see James et al. 2014). Branchiobdellidans were only found at one site in the River Gavenny

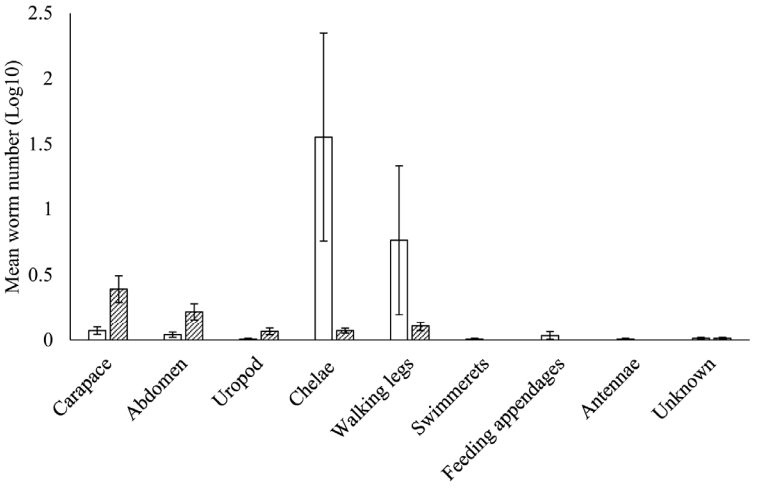

Figure 1. Mean ± SE (Log10) number of branchiodellidans of *Xironogiton victoriensis* (white bars) and *Cambarincola* aff. *okadai* (hatched bars) on different regions of the crayfish host, *Pacifastacus leniusculus*.

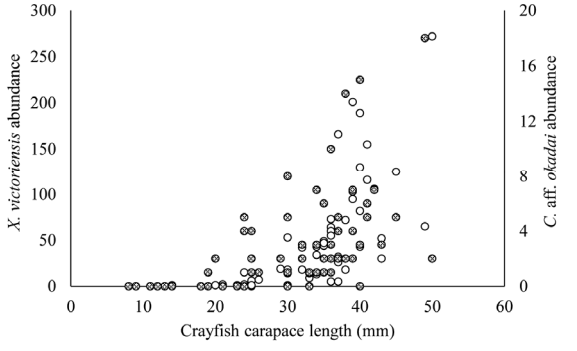

Figure 2. Correlation between crayfish size (carapace length, mm) and abundance of *Xironogiton victoriensis* (white circles) and *Cambarincola* aff. *okadai* (hatched circles).

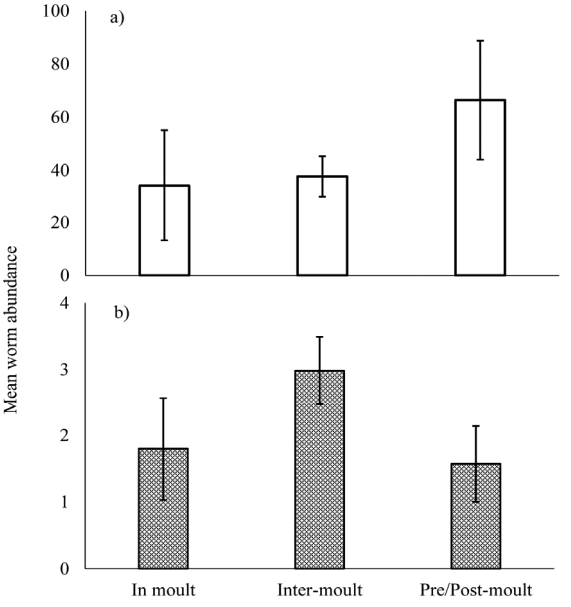

Figure 3. Mean (±SE) abundance of a) *Xironogiton victoriensis* and, b) *Cambarincola* aff. *okadai* on in moult, inter-moult and pre or post-moult crayfish.

(Ordnance Survey Grid Reference: SO308164), Abergavenny, Wales, on 18.5% of signal crayfish (5 out of 27) screened in 2012. A further 73 crayfish were collected in the five surveys conducted at the River Gavenny between April and June 2013. Two species of branchiobdellidans were found, subsequently identified through morphological and molecular analysis as *Xironogiton victoriensis* and *Cambarincola* aff. *okadai*. The prevalence of *X. victoriensis* and *C.* aff. *okadai* across these surveys were 75.34% and 71.23% respectively. Mean infection intensities were 52.93 (range: 1–272) and 3.88 (range: 1–18) for *X. victoriensis* and *C.* aff. *okadai* respectively.

Among infested hosts, co-infection with *X. victoriensis* and *C.* aff. *okadai* was common, with 75.41% of signal crayfish harbouring both species, but there was evidence of niche segregation with *X. victoriensis* found mainly on the chelae and *C.* aff. *okadai* mostly on the carapace (Figure 1). Total branchiobdellidan burden was positively correlated with crayfish size (carapace length, mm) for *X. victoriensis* (GLMM; $LRT_{1,65} = 100.13$, $P < 0.0001$) and *C.* aff. *okadai* ($LRT_{1,63} = 29.70$, $P < 0.0001$) (Figure 2). Branchiobdellidan burden was also influenced by moult stage for *X. victoriensis* (GLMM; $LRT_{3,65} = 10.94$, $P = 0.01$) and *C.* aff. *okadai* ($LRT_{3,63} = 15.40$, $P = 0.002$) (Figure 3). For *X. victoriensis,* worm burdens were highest on pre or post-moult crayfish and

lowest on crayfish in the process of moulting whereas for *C.* aff. *okadai* they were highest on inter-moult crayfish and lowest on pre or post-moult crayfish (although mean numbers of *C.* aff. *okadai* on pre and post-moult and in moult crayfish were similar) (Figure 3). For *C.* aff. *okadai*, branchiobdellidan burden was also significantly affected by crayfish sex/life stage (GLMM; $LRT_{1,63}$ = 6.45, P = 0.04) with juveniles having the fewest worms and males and females having similar higher numbers. No branchiobdellidans were found in the branchial chambers of dissected crayfish.

Morphological identification

Xironogiton victoriensis Gelder and Hall, 1990

Material examined: Gavenny River, Abergavenny, southeast Wales, on chelae and walking legs of signal crayfish, *Pacifastacus leniusculus*, coll. J. James: 6 specimens, 23.04.14 (NMW.Z.2014.012. 012-16); 4 specimens, 17.04.13 (NMW.Z.2014. 012.017); 9 specimens, 20.05.14 (NMW.Z.2014. 012.018); 13 specimens, 21.05.14 (NMW.Z.20 14.012.019); jaws, slide preparation (NMW.Z.20 14.012.020).

Description: Colour in life (Figure 4A-C) generally transparent to white, with green-brown gut and white reproductive organs discernable to varying extent; brown jaws visible through cuticle at peristomium-head junction. Fixed or preserved specimens opaque, white.

Exemplar live animal (Figure 4A) varying from short, anteriorly sub-cylindrical and posteriorly broad, flask-shape (total length 2.6 mm, maximum width 1.2 mm), to elongated pyriform (4.9 mm, 0.8 mm); segments 5–8 dorsally convex, ventrally flattened to concave with narrow skirt-like lateral margins.

Animals narcotized by exposure to $MgCl_2$ pyriform in shape (Figure 4B, C); when subsequently formalin fixed, short to moderately long (total length 1.6–4.3 mm, mean 3.2 mm, n=4), pyriform (maximum width 0.56–1.19 mm, mean 0.92 mm, n=3); length:width ratio 3.2–5.5, mean 4.3, n=3; head region:total length ratio 0.17–0.20, mean 0.185, n=4. Direct ethanol preserved specimens (Figure 5A) short (1.5–2.7 mm, mean 2.1 mm, n=28), broad flask shaped (width 0.56–1.44 mm, mean 1.05 mm, n=27); length:width ratio 1.6–3.0, mean 2.0, n=27; head region:total length ratio 0.18–0.25, mean 0.214, n=28.

Head region comprising peristomium and head, former delimited by distinct anterior constriction. Peristomium short, about a third as long as the head; upper lip usually bilobed, lower

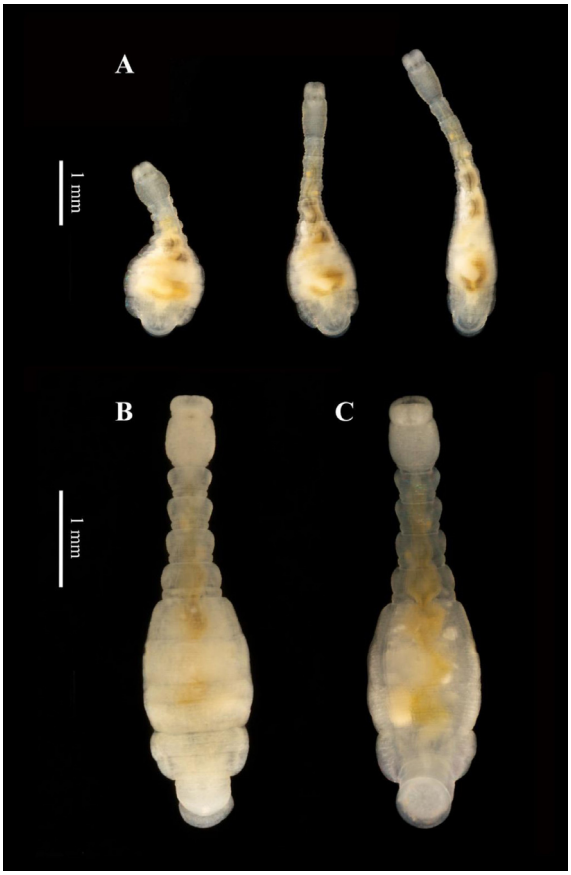

Figure 4. *Xironogiton victoriensis* (A) live animal, three dorsal views (NMW.Z.2014.012.013); (B, C) $MgCl_2$ narcotized animal, dorsal and ventral views (NMW.Z.2014.012.012).

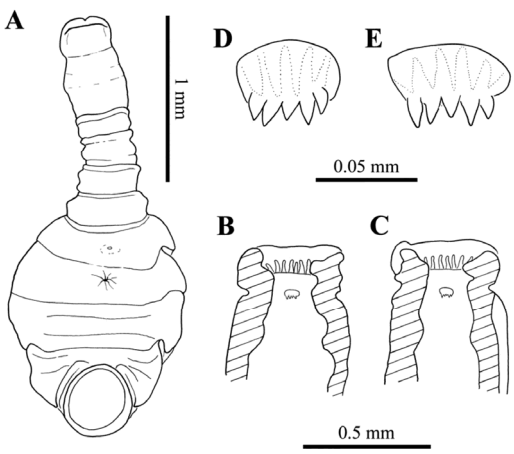

Figure 5. *Xironogiton victoriensis* (A) formalin fixed specimen, ventral view (NMW.Z.2014.012.013); (B, C) anterior pharyngeal region, frontal plane section, showing dorsal and ventral jaws and most oral papillae (NMW.Z.2014.012.012); (D, E) dorsal and ventral jaws, viewed from pharynx (NMW.Z.2014.012.013).

lip straight to broadly emarginate, surfaces adorned with small groups of stiff cilia; no tentacles. Dorsal jaws visible, with alternating dark brown-light brown longitudinal banding. Oral papillae difficult to discern, digitiform, 16, arising around mouth and anterior to the jaws (Figure 5B, C). Dorsal and ventral jaws similar, rounded rectangular plates with posteriorly directed teeth. Dental formulae exhibiting only slight variation: 5/4, 5/5 (Figure 5D, E) and 4/5 (Figure 5B, C) observed. Teeth unequal, middle tooth shortest, one lateral often a little more robust and curved.

Anterior nephridiopores not seen. Spermathecal pore mid-ventrally on segment 5, short clavate spermatheca visible through cuticle. Male genital pore on segment 6 more conspicuous, with slightly raised margins (Figure 5A). Anus conspicuous in live animals, opening on segment 10, dorsal to the sucker (Figure 4A, B). Posterior sucker a circular disc.

Remarks: The shape of the species varies greatly in life and with respect to the fixation or preservation procedure. Our morphometric analyses show that, while the relationship of the head region to overall length was similar (mean values of 18.8 and 21.4%), total length to width ratios differed markedly (mean values 4.3 vs 2.0) between $MgCl_2$ narcotized formalin fixed and direct ethanol (100%) preserved specimens.

The characteristics of the Welsh material correspond well with the original description from British Columbia, Canada (Gelder and Hall 1990) and subsequent records from Europe. The light-dark brown banding of the teeth was consistent with the appearance of the jaws photographed by Oberkofler et al. (2002: Figure 8) and Geasa (2014: Figure 4N). The first European record of *X. victoriensis* (as *Xironogiton instabilis* Moore, 1893; see Gelder et al. 2012) was from Sweden (Franzén 1962). Since then, the species has been found further south in Spain (Gelder 1999; Oscoz et al. 2010), Italy (Quaglio et al. 2001; Oberkofler et al. 2002; Gelder 2004), Hungary (Kovács and Juhász 2007), Austria (Nesemann and Neubert 1999 cited in Subchev 2008), France (Gelder et al. 2012) and potentially Finland (Kirjavainen and Westman 1999). Klobucar et al. (2006) stated that the species "is almost certainly to be found wherever the [signal] crayfish has been introduced." In terms of the current study *X. victoriensis* was only found in one signal crayfish population but we do not have details on the timing or county of origin of most British signal crayfish populations, so are unable to assess the relevance of our finding.

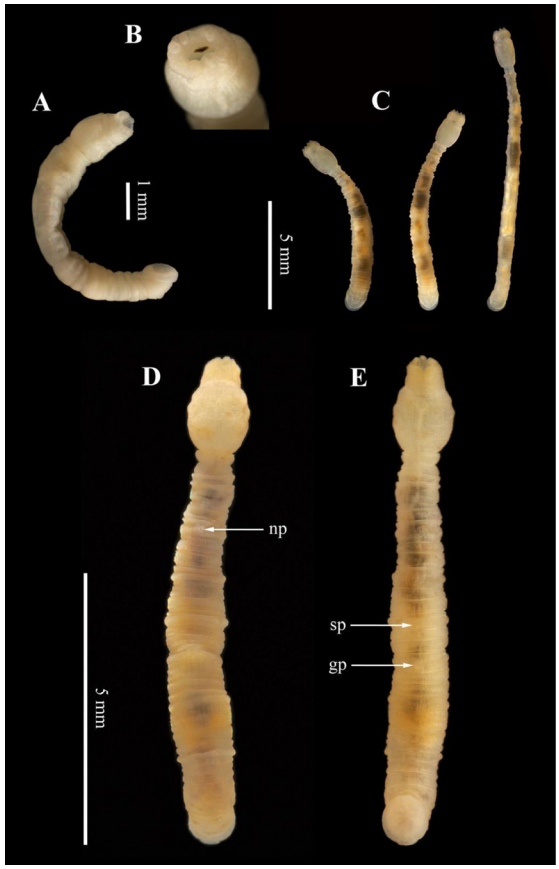

Figure 6. *Cambarincola* aff. *okadai* (A) 100% ethanol preserved specimen, lateral view; (B) same, anterior, oblique ventral view (A, B: NMW.Z.2014.012.001); (C) live animal, three dorsal views (NMW.Z.2014.012.008); (D, E) $MgCl_2$ narcotized animal, dorsal and ventral views (NMW.Z.2014.004). gp, male genital pore; np, nephridial pore; sp, spermathecal pore.

Cambarincola aff. *okadai* Yamaguchi, 1933

Material examined: Gavenny River, Abergavenny, southeast Wales, at the dorsal cephalothorax and abdomen of signal crayfish, *Pacifastacus leniusculus*, coll. J. James: 4 specimens, 17.04.13 (NMW.Z. 2014.012.001-2); 10 specimens, 23.04.14 (NMW. Z.2014.012.003-8); 14 specimens, 20.05.14 (NMW. Z.2014.012.009); 3 cocoons, 23.04.14 (NMW. Z.2014.012.010); jaws, slide preparation (NMW. Z.2014.012.011).

Description: Colour in life (Figure 6C-E) yellow-white, with paler white-transparent head and posterior sucker regions; dark gut contents visible for most of body length. Brown jaws indistinctly visible through cuticle at peristomium-head junction (Figure 4C); more clearly seen through mouth opening (Figure 6B). Fixed or preserved specimens opaque, white (Figure 6A).

Exemplar live animal sub-cylindrical (Figure 6C), varying from short and stout (total length 7.5 mm, maximum body segment width 1.07 mm) to long and narrow (13.2 mm, 0.80 mm). Maximum observed length of other animals in petri dish, 17 mm.

Narcotized animals sub-cylindrical (Figure 6D, E); when subsequently formalin fixed, moderately long (4.1–8.2 mm, mean 7.1 mm, n=8) and wide (0.61–1.20 mm, mean 0.96 mm, n=5); length:width ratio 6.6–7.7, mean 7.0, n=5; head region:total length ratio 0.18–0.22, mean 0.194, n=8. Ethanol preserved specimens somewhat dorso-ventrally flattened (Figure 6A, 7A), short to moderately long (4.6–8.3 mm; mean 6.0 mm, n=20) and stout (width 1.06–1.67 mm, mean, 1.35 mm, n=19); length:width ratio 3.9–5.2, mean 4.4, n=19; head region:total length ratio 0.18–0.24, mean 0.215, n=20.

Head region comprising peristomium and head, former delimited by distinct anterior constriction. Peristomium short, about 30% of the head length. Peristomial shape very variable in live animals, from narrow cylindrical to anteriorly wide funnel-like; in alcohol preserved specimens, often more constricted and anteriorly tapering, somewhat conical. Upper lip bearing 2 pairs of short tentacles, medial gap between pairs larger than separation of tentacles in each pair (Figure 6B, C), Shape of tentacles variable relative to degree of contraction or expansion of peristomium, ranging from blunt rounded lobes (Figure 6A, B, D, E, 7A) to more pointed conical (Figure 6C). When peristomium expanded and anteriorly flared in live animals, or when oral papillae are extruded (Figure 7B), the tentacles become reduced and almost disappear, with only short blunt tips remaining. Ventral lip bilobed with 2 broadly rounded lobes (Figure 6B, E; 7A, B, D). Weakly defined lobes laterally (2–3, usually 3), and even more indistinct 'lobes' between each tentacle pair (2–3) and between tentacles in each pair (1–2). Head cylindrical to barrel-shaped, wider than anterior segments in live animals.

Oral papillae difficult to discern unless protruded (Figure 7B) or head region dissected (Figure 7C, D). Papillae triangular, 16, arising around mouth and anterior to the jaws. Jaws subequal, broad (up to ca. 220 µm across), triangular, each with a single large bluntly triangular tooth and 2 pairs of small secondary teeth laterally, on exposed oral (dorsal) surfaces; dentition 5/5. Secondary teeth sharper, more projecting and readily observed on pale brown 'younger' jaws; 'older' jaws very dark brown, robust with low bluntly rounded secondary teeth (Figure 7E, F). Secondary teeth can often only be confirmed through examination using a compound microscope; jaws appearing unidentate when viewed using a stereo-zoom dissecting microscope.

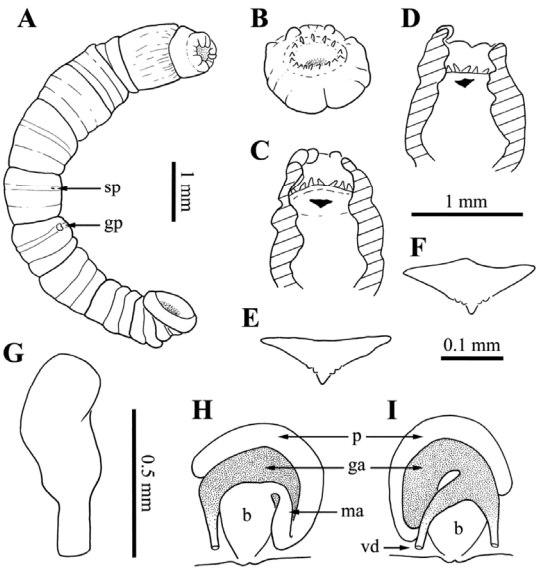

Figure 7. *Cambarincola* aff. *okadai* (A) 100% ethanol preserved specimen, lateral view (NMW.Z.2014.012.001); (B) formalin fixed specimen, anterior with 16 oral papillae exposed, oblique ventral view (NMW.Z.2014.012.003); (C, D) anterior pharyngeal region, frontal plane section, showing dorsal and ventral jaws and most oral papillae; (E, F) dorsal and ventral jaws, viewed from pharynx; (G) spermatheca; (H, I) male genitalia, left and right lateral views (C-I: NMW.Z.2014.001). b, bursa; ga, glandular atrium; gp, male genital pore; ma, muscular atrium; p, prostate; sp, spermathecal pore; vd, vas deferens.

Body segments primarily triannulate, though further lesser annulations also occur. Low thickened ring additionally present on each segment in live animals (Figure 6C-E), most obvious on segments 4–8; pronounced raised dorsal ridges absent. Thickened rings indistinct on fixed and preserved specimens.

A single mid-dorsal nephridiopore occurs on segment 3 (Figure 6D). The nephridiopore sometimes inconspicuous, but often revealed through the use of Methyl Green or Shirlastain A staining. Segment 4 with a long strap-like nephridium, distal part lying transversely above the gut at posterior of segment, proximal part passing ventrally and anteriorly into segment 3. Spermathecal pore mid-ventrally on segment 5. Male genital pore on segment 6 more conspicuous, often with clearly protruding margins (Figure 6E, 7A). Posterior sucker a large circular and concave disc.

Spermatheca variable, ranging from having a short duct leading to a longer distally rounded cylindrical structure (Figure 7G) to a long narrow duct and a wide globular distal bulb. Male genitalia with long tubular prostate gland positioned above the glandular atrium, distal region of latter varying from a Y-shape, curving round the bursa (Figure 7H, I), to a more triangular, less indented structure. Methyl Green staining deepest in glandular atrium, moderate in bursa and weak in prostate.

Stalked cocoons (from crayfish cuticle) transparent with variable amount of red-brown stellate ornamentation, each containing a single larva. Larvae active, moving within cocoon, hatching from the top when sufficiently developed.

Remarks: The total length to width relationship of this large species varies greatly in life and with respect to the fixation or preservation procedure. While the head region to overall length relationship was similar (mean values of 19.4 and 21.5%), length to width ratios were quite distinct (mean values 7.0 vs 4.4) between $MgCl_2$ narcotized formalin fixed and direct ethanol (100%) preserved specimens.

The upper peristomial lip of the Welsh species bore 4 long dorsal tentacles, a character shared with 4 species from North America: *C. okadai* Yamaguchi, 1933 (a North American introduction to Japan), *C. macrocephalus* Goodnight, 1943 (Wyoming), *C. fallax* Hoffman, 1963 (Virginia), and *C. holti* Hoffman, 1963 (Kentucky). Holt (1974) redescribed *Triannulata montana* Goodnight, 1940 (Washington) and transferred it to *Cambarincola*, while Gelder and Ohtaka (2000) later synonymized it with *C. okadai*. Several other species – *C. philadelphicus* (Leidy, 1851: Pennsylvania), *C. chirocephalus* Ellis, 1919 (Missouri), *C. ingens* Hoffman, 1963 (Virginia), and *C. gracilis* Robinson, 1954 (see Holt 1981) – have 4 small lobes on the dorsal lip but, according to Hoffman (1963), this condition "in no way approximates the conspicuous tentaculation of *fallax* and some other species."

Other consistent features of the Welsh material were a lower lip bearing 2 large broadly rounded lobes, and large triangular jaws armed with a single strong distal tooth and 2 pairs of small dorso-lateral secondary teeth. However, the specimens exhibited intra-specific variations in the morphology of the spermatheca and male reproductive apparatus that encompassed some of the inter-specific differences used in distinguishing the 'tentaculate' species in the comprehensive (though sometimes ambiguous) key to 48 species of *Cambarincola* presented by Holt and Opell (1993).

Hence a distally bilobed to slightly emarginate triangular glandular atrium, together with a prostate of similar or slightly shorter length, could be considered common to *C. okadai*, *C. macrocephalus*, *C. fallax*, *C. holti*, *C. philadelphicus* and *C. chirocephalus*. *Cambarincola ingens* is distinguished by having an additional long sinuous or coiled distal extension to the prostate (Hoffman 1963: Figure 34; Holt and Opell 1993: Figure 95). All these species have spermathecal morphologies encompassed in the variation observed in the Welsh specimens, except *C. holti* that has an additional tubular and highly glandular distal extension (Hoffman 1963: figure 60; Holt and Opell 1993: Figure 89). *Cambarincola gracilis* differs from all the above in having a conspicuously shorter prostate gland (see Holt 1981: Figure 2C).

Four species (*C. philadelphicus*, *C. fallax*, C. *chirocephalus* and *C. gracilis*) are reported to possess "prominent dorsal ridges" (Holt and Opell 1993) in preserved specimens. These dorsal ridges may actually encircle the segments (e.g., in *C. gracilis*; see Gelder et al. 2012: Figure 1). By contrast, the low thickened segmental rings, observed in the live Welsh animals, were absent or scarcely visible in non-narcotized ethanol preserved specimens.

The morphology of the Welsh species most closely resembles that of the remaining two species, *C. okadai* and *C. macrocephalus*, though the latter could be separated from both by having a head region "approaching $^1/_3$ entire body in size" (Holt 1981) and this feature was used as an early separator in Holt and Opell's key (1993). There are nevertheless similarities in the body annulation pattern, peristomial tentacles and lobes, and jaws. Considering first the body annulations; Goodnight (1940) originally created a new genus *Triannulata*, for *T. magna* and *T. montana* (latter currently a junior synonym of *C. okadai*), in acknowledgement of the secondary annulations evident on each segment. Gelder and Ohtaka (2000) confirmed the presence of triannulate segments in their redescription of *C. okadai*. Goodnight (1943) in his original description of *C. macrocephalus* referred to the major annulations of the body segments as "not elevated over minor annulations" and, in his re-description of the holotype, Hoffman (1963) noted that some of the anterior segments "notably from III to VI, are quite distinctly tripartite." However, Holt (1981) dismissed Hoffman's observation as a misinterpretation of a slide preparation.

Regarding the peristomial structures, the infrequently reported *C. macrocephalus* was

recorded (Hoffman 1963) as having "four distinct slender submarginal tentacles" dorsally and as being "broadly bilobed" ventrally. This is very similar to the account of the peristomium in the original description of *C. okadai*; the dorsal part "having four distinct digitiform appendages, while the ventral part is thick and slightly bilobed" (Yamaguchi 1933). Its junior synonym *T. montana* was described as having its peristomium divided in to "twelve lobes (four dorsal, four ventral, and four lateral) which may be extended into tentacular appendages, dorsal longer than ventral or lateral" (Goodnight 1940). This arrangement was confirmed by Holt (1974), who distinguished the tentacles of *C. montanus* n.comb. from the lobes, "4 dorsal tentacles, 2 lateral lobes either side and 4 ventral lobes." Holt also remarked that variations in the length of the tentacles were of no consequence, and that oral papillae were not detectable. In a later account, Holt (1981) referred to the lateral and ventral lobes as "prominent". Gelder and Ohtaka (2000) redescribed *C. okadai* and reported the peristomium as having a "dorsal lip with four distinct lobes (l) (or tentacles), two pairs of lateral lobes, and a ventral lip (v) consisting of a pair of short lobes laterally and a central portion with a slight median incision." In the Welsh material, we found a ventral lip of 2 broadly rounded lobes and above these, either side, up to 3 smaller, weakly defined, lateral lobes. Should the most inferior of these lateral lobes be considered 'ventral' then the Welsh specimens would be in complete agreement with Gelder and Ohtaka (2000: Figure 1B, reproduced in Gelder et al. 2012: Figure 6).

Literature accounts of jaw dentition in *Cambarincola* are often quite variable and this may reflect natural variation, differences between young and old jaws, inadequate microscopical examination, differences between populations, or a compounding of different species. Goodnight (1943) discovered *C. macrocephalus* on the Pilose Crayfish, *Pacifastacus gambelii* (Girard, 1852); mature worms were up to 4 mm long. The jaws were described as "large triangular blocks of chitin terminating in sharp tooth. Without lateral teeth but margin uneven. Width of jaws at base 400 µm." Hoffman (1963) re-examined the holotype and, with some reservation, some material from a new locality in Idaho. Preserved worms were up to 4.8 mm (0.8 mm wide) with a head region up to 1.1 mm wide, and the jaws large and robust with 3/3 dentition. Holt (1981) emended Hoffman's description and cited preserved specimens up to 5.6 mm long and 1.4 mm wide. The jaws were interpreted as having a single tooth, "though there appear to be lateral teeth on the jaws of younger animals" and the latter were illustrated (Holt 1981: Figure 3C) with a 3/3 dentition. Recently, Geasa (2014) recorded *C. macrocephalus* among the branchiobdellidans found on signal crayfish (*Pacifastacus leniusculus*) from three out of seven locations in British Columbia, western Canada. Length was recorded as up to 7 mm, and the jaws described as having 1/1 dentition.

In the original description of *C. okadai*, Yamaguchi (1933) described worms up to 7 mm long and 0.8 mm wide, from signal crayfish introduced to Japan. The jaws were triangular with a large conical tooth and 2 small denticles either side. Drawings of the jaws by Yamaguchi (1933: Figure 2A, B) and in the redescription by Gelder and Ohtaka (2000: Figure 1C, D; see also Gelder et al. 2012, Figure 7) are morphologically identical to those found in young Welsh specimens. Gelder and Ohtaka (2000) noted a discrepancy regarding total length in the type material and found it to be up to 4.7 mm; jaws were 95–130 µm wide. Despite this, Gelder et al. (2012: Figure 2) presented a photograph of a 7 mm long adult from France. Goodnight (1940) described the triangular jaws of *T. montana* as measuring 250 µm across in a worm 5 mm long (1.25 mm wide); dentition 7/5, each with a longer median tooth and smaller lateral ones. Holt (1974) subsequently reported *C. montanus* n.comb. as being up to 6.3 mm long and 1.0 mm wide; dentition usually 1/1, but 5/5 in younger, though large, specimens from the type locality. In a later paper, Holt (1981) inexplicably gave the dentition as 1/2, and 5/5 in immature forms. However, Gelder and Hall (1990) figured jaws of sexually mature specimens from British Columbia with 5/5 dentition. An SEM image of the peristomial tentacles and lobes in *C. montanus* from British Columbia (Geasa 2014: Figure 6C) is strikingly similar to their appearance in Welsh *Cambarincola* material.

Consideration of all the morphological information shows that the Welsh specimens have the closest affinity with *C. okadai*, but are noticeably larger (at least 8.3 mm long, preserved; live animals extending to at least 17 mm), with larger jaws (195–220 µm wide). This jaw size approaches that indicated for *T. montana* and raises questions about its synonymy with *C. okadai*. Indeed, *C. okadai* as currently understood could conceivably encompass an even larger number of cryptic species. Misidentification of branchiobdellidans is common. For example, Williams et al. (2013)

found a 49% error in Genbank sequences, and their molecular analyses indicated that *C. philadelphicus sensu lato* comprised at least four cryptic (and unrelated) taxa. A similar situation may well exist in a *C. okadai–C. montanus* group and this can only be resolved by a combined morphological and molecular study of material from western USA and Canada, and Japan.

Genetic analysis

The mitochondrial COI partial sequence (653 bp) obtained from the *X. victoriensis* specimens (Genbank Accession Number KT025254) was 99% similar to *Xironogiton victoriensis*, Genbank Accession Number: JQ821631.1, differing at base pairs 265 (G: A), 340 (C: T) and 581 (T: C). A 99% sequence match for *X. victoriensis* specimens was also obtained for *Sathrodrilus attenuatus* (AF310719.1), differing at base pairs 300 (G: A), 450 (G: A) and 523 (C: T), which Williams et al. (2013) indicated was a misidentified *X. victoriensis* specimen. The best quality sequence obtained for the *Cambarincola* aff. *okadai* species was a 598 bp fragment (Genbank Accession Number KT025253) with 1 nucleotide ambiguity (at base pair 134) which was a 91% match to *Cambarincola montanus* (synonymized with *C. okadai* by Gelder and Ohtaka 2000), Genbank Accession Number: AF310711.1.

Discussion

We provide the first record of non-native branchiobdellidans on invasive crayfish in the British Isles. Both identified branchiobdellidan species, *Xironogiton victoriensis* and *Cambarincola* aff. *okadai* had a relatively high prevalence of 75.34% and 71.23% respectively in the infected signal crayfish (*Pacifastacus lenisuculus*) population. Further studies are required to determine the potential community level consequences of this symbiotic relationship.

Xironogiton victoriensis is native to Canada (Gelder and Hall 1990) and within Europe was first reported in Sweden (Franzén 1962) and subsequently Spain (Gelder 1999; Oscoz et al. 2010; Vedia et al. 2014), Italy (Quaglio et al. 2001; Oberkofler et al. 2002), Hungary (Kovács and Juhász 2007), Austria (Nesemann and Neubert 1999 cited in Subchev 2008), France (Laurent 2007; Subchev 2008; Gelder et al. 2012) and potentially Finland (Kirjavainen and Westman 1999). It should be noted that Nesemann and Neubert (1999 cited in Subchev 2008) and Kovács and Juhász (2007) all identified their *Xirongiton* specimens as *X. instabilis* but it is almost certain that these are samples of *X. victoriensis* that were misidentified (Subchev 2008). In Europe, *X. victoriensis* are mostly found on North American signal crayfish (Franzén 1962; Kirjavainen and Westman 1999; Gelder 1999; Quaglio et al. 2001; Oberkofler et al. 2002; Kovács and Juhász 2007; Laurent 2007; Subchev 2008; Oscoz et al. 2010; Gelder et al. 2012), which they infest in their native range (Gelder and Hall 1990). In Spain, *X. victoriensis* have however, recently been recovered from Louisiana red swamp crayfish (*Procambarus clarkii*) with which they do not naturally co-exist (Vedia et al. 2014). Because of the generalist host range of *X. victoriensis* all invasive crayfish species in the UK should be considered as a potential host, and thus transmission pathway, for this symbiont.

Cambarincola okadai, the closest identified relative of *C.* aff. *okadai*, is native to North America (Yamaguchi 1933) and within Europe has only been recorded on signal crayfish from France (Gelder et al. 2012). As the specimens of *C. okadai* from France were identified only through morphological techniques (Gelder et al. 2012) it is possible that they are actually the same species as the *C.* aff. *okadai* described in the current paper; the genetic analysis of French *C. okadai* would be needed to test this. Additionally, the specimens of *C.* aff. *okadai* described in the current study exhibit some morphological similarity with *C. macrocephalus* (see Goodnight 1943). However, as no genetic sequence is currently available for the latter, we are unable to determine whether this is the first record of a new species of branchiobdellidan or alternatively a range extension of an existing one. Nevertheless, the current study is the first report of *C.* aff. *okadai*, as well as *X. victoriensis*, in the British Isles, representing an increase in the global spread of branchiobdellidans.

In the current study branchiobdellidans were only present at one of the 9 sites positive for invasive signal crayfish. Further, there were no reports of branchiobdellidans in any of the 7,161 signal crayfish records analysed from the UK national crayfish database, CrayBase (James et al. 2014). This includes surveys conducted in the same river catchment, the Usk, as the branchiobdellidan infested population in the River Gavenny (Slater personal communication). The absence of branchiobdellidans in nearby signal crayfish populations suggests that their introduction into the Gavenny occurred fairly recently. Furthermore, records of native white clawed crayfish (*Austro-*

potamobius pallipes) in the Gavenny from 2000 implies that the introduction of signal crayfish into this river may also have occurred as recently as the last decade or two since natives are typically displaced by signal crayfish.

Potential ecological effects on British crayfish, due to *X. victoriensis* and *C.* aff. *okadai* introductions, are unknown as the relationship between crayfish and branchiobdellidans varies from parasitism (e.g. Rosewarne et al. 2012) to commensalism (e.g. Keller 1992) to mutualism (e.g. Brown et al. 2002, 2012; Lee et al. 2009) depending on the worm and crayfish species, environmental conditions (Lee et al. 2009) and branchiobdellidan density (Brown et al. 2012). Branchiobdellidan densities on British crayfish ranged from 1–272 for *X. victoriensis* and 1–18 for *C.* aff. *okadai* and were influenced by crayfish size, moult status and, for *C.* aff. *okadai,* sex. As observed in previous studies (e.g. Keller 1992; Brown and Creed 2004; Skelton et al. 2014) the abundance of both branchiobdellidans, increased with crayfish size. Larger crayfish have a greater surface area available for branchiobdellidan colonization and moult less frequently which is important as *X. victoriensis* and *C.* aff, *okadai* reside on the exoskeleton of the crayfish (Koepp 1975). Additionally, larger crayfish may also exhibit reduced grooming responses to branchiobdellidans than smaller individuals, potentially contributing to the frequently observed relationship between crayfish size and branchiobdellidan abundance (Skelton et al. 2014). Age-specific variation in grooming has, however, only been observed in branchiobdellidans known to clean epibionts from crayfish gills (Skelton et al. 2014). Such ontogenetic changes in grooming behaviour are predicted to reflect age related shifts in the cost-benefits of the symbiosis to the crayfish host (Skelton et al. 2014). We did not, however, observe either *X. victoriensis* or *C.* aff. *okadai* in the branchial chambers of crayfish, and so there is no evidence that they are involved in a cleaning symbioses with the host. Indeed we found that moult phase had a significant effect on the abundance of both worm species. As we were, however, not able to distinguish between pre and post-moult crayfish we do not make any specific predictions regarding the relationship between crayfish moult stage and branchiobdellidan abundance. *C.* aff. *okadai* abundance was also influenced by crayfish sex/life stage, being lower on juvenile crayfish than either adult males or females. This may because of the smaller area available for colonization by *C.* aff. *okadai,* which are relatively large branchiobdellidans, on juvenile crayfish. We, however, found no evidence of a significant interaction between crayfish size and sex/life stage in explaining variation in *C.* aff. *okadai* abundance. Therefore increased moulting frequency of juveniles may be more important that size in determining *C.* aff. *okadai* abundance. .

We observed a high level of co-infection between *X. victoriensis* and *C.* aff. *okadai*, therefore it is likely that crayfish migrating from the infected population will harbour both species. Inter-specific competition may alter the effect of one or both branchiobdellidan species on the crayfish host by causing worms to switch their micro-habitat or feeding preference. We found evidence of *X. victoriensis* and *C.* aff. *okadai* micro-habitat niche segregation on crayfish hosts but, due to a paucity of uninfected crayfish, it was not possible to determine if this was the result of inter-specific competition. We found no worms of either species in the branchial chamber of dissected infected crayfish although only a small number of crayfish were examined (n = 10).

Overall, the current study documents the presence of two novel symbiotic annelids in the British Isles, one of which may be a previously undescribed species. Future work involving comparative sequencing of mitochondrial CO-I from *C. okadai* and *C. macrocephalus* populations is needed to elucidate the identity of *C.* aff. *okadai*. This highlights the need to improve the current sequence data available for branchiobdellidans, something that has already been demonstrated by Williams et al. (2013). Experiments assessing the nature of the relationship between crayfish and *X. victoriensis* and *C.* aff. *okadai,* are needed to assess the potential consequences of branchiobdellidan infection for both invasive signal and endangered native crayfish in Britain.

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References

Bishop JE (1968) An ecological study of the Branchiodellid commensals of some mid western Ontario crayfish. *Canadian Journal of Zoology* 46: 835–843, http://dx.doi.org/10.1139/z68-119

Brown BL, Creed RP (2004) Host preference by an aquatic ectosymbiotic annelid on 2 sympatric species of host crayfishes.

Journal of the North American Benthological Society 23: 90–100, http://dx.doi.org/10.1899/0887-3593(2004)023<0090:HPBAAE>2.0.CO;2

Brown BL, Creed RP, Dobson WE (2002) Branchiobdellid annelids and their crayfish hosts: are they engaged in a cleaning symbiosis? *Oecologia* 132: 250–255, http://dx.doi.org/10.1007/s00442-002-0961-1

Brown BL, Creed RP, Skelton J, Rollins MA, Farrell KJ (2012) The fine line between mutualism and parasitism: complex effects in a cleaning symbiosis demonstrated by multiple field experiments. *Oecologia* 170: 199–207, http://dx.doi.org/10.1007/s00442-012-2280-5

Crawley MJ (2007) Statistical modelling. In: Crawley MJ (ed), The R book. Wiley, England, UK, pp 323–386, http://dx.doi.org/10.1002/9780470515075.ch9

Ellis MM (1919) The branchiobdellid worms in the collections of the United States National Museum, with descriptions of new genera and species. *Proceedings of the United States National Museum* 55: 241–265, http://dx.doi.org/10.5479/si.00963801.55-2267.241

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299

Franzén Å (1962) Notes on the morphology and histology of *Xironogiton instabila* (Moore, 1893) (Fam. Branchiobdellidae) with special reference to the muscle cells. *Zoologiska Bidrag Fran Uppsala* 35: 369–383

Geasa NMSH (2014) Observation of Branchiobdellidae (Clitellata, Annelida) on Freshwater Crayfish *Pacifastacus leniusculus* in British Columbia, Canada. *Sci-Afric Journal of Scientific Issues Research and Essays* 2: 94–107

Gelder SR (1996) A review of the taxonomic nomenclature and a checklist of the species of the Branchiobdellae (Annelida: Clitellata). *Proceedings of the Biological Society of Washington* 109: 653–663

Gelder SR (1999) Zoogeography of branchiobdellidans (Annelida) and temnocephalidans (Platyhelminthes) ectosymbiotic on freshwater crustaceans, and their reactions to one another *in vitro*. *Hydrobiologia* 406: 21–31, http://dx.doi.org/10.1023/A:1003755630718

Gelder SR, Hall LA (1990) Description of *Xironogiton victoriensis* n.sp. from British Columbia, Canada, with remarks on other species and a Wagner analysis of *Xironogiton* (Clitellata: Branchiobdellida). *Canadian Journal of Zoology* 68: 2352–2358, http://dx.doi.org/10.1139/z90-326

Gelder SR, Ohtaka A (2000) Redescription and designation of lectotypes of the Northern American *Cambarincola okadai* Yamaguchi, 1933 (Annelida: Clitellata: Branchiobdellidae). *Proceedings of the Biological Society of Washington* 113: 1089–1095

Gelder SR, Parpet JF, Quaglio F (2012) First report of two North American branchiobdellidans (Annelida: Clitellata) or crayfish worms on signal crayfish in Europe with a discussion of similar introductions into Japan. *Annales de Limnology – International Journal of Limnology* 48: 315–322, http://dx.doi.org/10.1051/limn/2012021

Goodnight CJ (1940) The branchiobdellidae (Oliochaeta) of North American crayfishes. In: Buchhole JH, Tanner FW, Van Cleave HJ (eds), Illinois biological monographs volume XVII, University of Illinois, Urbana, Illinois, USA, pp 5–75

Goodnight CJ (1943) Report on a collection of branchiobdellids. *J. Parasitology* 29: 100–102, http://dx.doi.org/10.2307/3272943

Govedich FR, Bain BA, Moser WE, Gelder SR, Davies RW, Brinkhurst RO (2009) Annelida (Clitellata): Oligochaeta, Branchiobdellida, Hirudinida, and Acanthobdellida. In: Thorp JH, Covich AP (eds), Ecology and classification of North American freshwater invertebrates 3rd edition, Academic press, pp 385–410

Harper GL, King RA, Dodd CS, Harwood JD, Glen DM, Bruford MW, Symondson WOCS (2005) Rapid screening of invertebrate predators for multiple prey DNA targets. *Molecular Ecology* 14: 819–827, http://dx.doi.org/10.1111/j.1365-294X.2005.02442.x

Hoffman RL (1963) A revision of the North American annelid worms of the genus *Cambarincola* (Oligochaeta: Branchiobdellidae). *Proceedings of the United States National Museum* 114: 271–371, http://dx.doi.org/10.5479/si.00963801.114-3470.271

Holdich DM, Reeve ID (1991) Distribution of freshwater crayfish in the British Isles, with particular reference to crayfish plague, alien introduction and water quality. *Aquatic Conservation: Marine and Freshwater Ecosystems* 1: 139–158, http://dx.doi.org/10.1002/aqc.3270010204

Holt PC (1974) An emendation of the genus *Triannulata* with the assignment of *Triannulata montana* to *Cambarincola clitellata* branchiobdellida. *Proceedings of the Biological Society of Washington* 87: 57–72

Holt PC (1981) A resumé of members of the genus *Cambarincola* (Annelida, Branchiobdellida) from the Pacific drainage of the United States. *Proceedings of the Biological Society of Washington* 94: 675–695

Holt PC, Opell BD (1993) A Checklist of and illustrated key to the genera and species of the Central and North American Cambarincolidae (Clitellata: Branchiobdellida). *Proceedings of the Biological Society of Washington* 106: 251–259

James J, Slater FM, Cable J (2014) A.L.I.E.N. databases: Addressing the Lack In Establishment of Non-natives databases. *Crustaceana* 87: 1192–1199, http://dx.doi.org/10.1163/15685403-00003329

Keller TA (1992) The effect of the branchiobdellid annelid *Cambarincola fallax* on the growth rate and condition of the crayfish *Orconectes rusticus*. *Journal of Freshwater Ecology* 7: 165–171, http://dx.doi.org/10.1080/02705060.1992.9664681

Kirjavainen J, Westman K (1999) Natural history and development of the introduced signal crayfish, *Pacifastacus leniusculus,* in a small, isolated Finnish lake, from 1968 to 1993. *Aquatic Living Resources* 12: 387–401, http://dx.doi.org/10.1016/S0990-7440(99)00110-2

Koepp SJ (1975) Effects of host ecdysis on population structure of the epizootic branchiobdellid Cambarincola vitrea. *Science of Biology Journal* 1:39–42

Kovács T, Juhász P (2007) Data to the distribution of crayfish worms (Branchiobdellidae) in Hungary. *Folia Historico-Naturalia Musei Matraensis* 31: 77–79

Kozubíková E, Filipová L, Kozák P, Ďuriš Z, Martín MP, Dieguez-Uribeondo J, Oidtmann B, Petrusek A (2009) Prevalance of the crayfish plague pathogen *Aphanomyces astaci* in invasive American crayfishes in the Czech Republic. *Conservation Biology* 23: 1204–1213, http://dx.doi.org/10.1111/j.1523-1739.2009.01240.x

Klobucar GIV, Maguire I, Gottstein S, Gelder SR (2006) Occurrence of Branchiobdellida (Annelida: Clitellata) on freshwater crayfish in Croatia. *Annales de Limnologie – International Journal of Limnology* 42: 251–260, http://dx.doi.org/10.1051/limn/2006026

Laurent PJ (2007) A French population of *Pacifastacus leniusculus* bears the North American parasite branchiobdellidan ectosymbionts *Xironogiton victoriensis*. *Crayfish News* 29: 5–6

Lee JH, Won Kim T, Choe JC (2009) Commensalisom or mutualism: conditional outcomes in a branchiobdellid-crayfish symbiosis. *Oecologia* 159: 217–224, http://dx.doi.org/10.1007/s00442-008-1195-7

Leeke CJ, Price A (1965) *Branchiobdella astaci* in Reading. *The Reading Naturalis*t 17: 18–20

Leidy J (1851) Contributions to Helminthology. *Proceedings of the Academy of Natural Sciences Philadelphia* 5: 205–209

Longshaw M (2011) Diseases of crayfish: A review. *Journal of Invertebrate Pathology* 106: 54–70, http://dx.doi.org/10.1016/j.jip.2010.09.013

Mackie ASY, Gobin J (1993) A review of the genus *Johnstonia* Quatrefages, 1866 (Polychaeta: Maldanidae), with a description of a new species from Trinidad, West Indies. *Zoologica Scripta* 22: 229–241, http://dx.doi.org/10.1111/j.1463-6409.1993.tb00354.x

Oberkofler B, Quaglio F, Fureder L, Fioravanti ML, Giannetto S, Morolli C, Minelli G (2002) Species of Branchiobdellidae (Annelida) on freshwater crayfish in south Tyrol (northern Italy). *Bulletin Français de la Pêche et de la Pisciculture* 367: 777–784, http://dx.doi.org/10.1051/kmae:2002065

Oscoz J, Tomás P, Durán C (2010) Review and new records of non-indigenous freshwater invertebrates in the Ebro River basin (Northeast Spain). *Aquatic Invasions* 5: 263–284, http://dx.doi.org/10.3391/ai.2010.5.3.04

Petersen ME (1998) *Pholoe* (Polychaeta: Pholoidae) from northern Europe: a key and notes on the nearshore species. *Journal of the Marine Biological Association of the UK* 78: 1373–1376, http://dx.doi.org/10.1017/S002531540004457X

Quaglio F, Fioravanti ML, Gelder SR, Giannetto S, Trentini M, Nobile L, Maxia M, Morolli C (2001) Infestation of the branchiobdellidan, *Xironogiton victoriensis* (Annelida: Clitellata), on the signal crayfish (*Pacifastacus leniusculus*) from Auenbachl Creek, Alto Adige/Süd Tyrol, Italy. *Freshwater Crayfish* 13: 274–279

Rogers D, Hoffmann R, Oidtmann B (2003) Diseases in selected *Austropotamobius pallipes* populations in England. In: Proceedings of a conference on the management and conservation of crayfish. Nottingham, UK, 7 November 2002. Environment Agency, Bristol, UK, pp 169–174

Robinson DA (1954) *Cambarincola gracilis* sp. nov., a branchiobdellid oligochaete commensal on western crayfishes. *International Journal for Parasitology* 40: 466–469, http://dx.doi.org/10.2307/3273899

Rosewarne PJ, Mortimer RJG, Dunn AM (2012) Branchiobdellidan infestation on endangered white clawed crayfish (*Austropotamobius pallipes*) in the UK. *Parasitology* 139: 774–780, http://dx.doi.org/10.1017/S003118201100240X

Schrimpf A, Maiwald T, Vrålstad T, Schulz HK, Śmietana O, Schulz R (2013) Absence of the crayfish plague pathogen (*Aphanomyces astaci*) facilitates coexistence of European and American crayfish in central Europe. *Freshwater Biology* 58: 1116–1125, http://dx.doi.org/10.1111/fwb.12112

Skelton J, Creed RP, Brown B (2014) Ontogenetic shift in host tolerance controls initiation of a cleaning symbiosis. *Oikos* 123: 677–686, http://dx.doi.org/10.1111/j.1600-0706.2013.00963.x

Subchev MA (2008) Branchiobdellida (Annelida: Clitellata) found in the crayfish and annelid collections of the French National Museum of Natural History (Paris), and on recently collected crayfishes from France. *Acta Zoologica Bulgarica* 60: 233–237

Thomas R, Vaughan I, Lello J (2013) Data analysis with R statistical software, a guide book for scientists. Eco-explore, Newport printing, Wales, UK

Torchin ME, Lafferty KD, Kuris AM (2002) Parasites and marine invasions. *Parasitology* 124: 137–151, http://dx.doi.org/10.1017/S0031182002001506

Truett GE, Heeger P, Mynatt RL, Truett AA, Walker JA, Warman ML (2000) Preparation of PCR – quality mouse genomic DNA with hot sodium and tris (HotSHOT). *Biotechniques* 29: 52–54

Unestam T, Weiss DW (1970) The host-parasite relationship between freshwater crayfish and the crayfish disease fungus *Aphanomyces astaci*: response to infection by a susceptible and a resistant species. *Journal of General Microbiology* 60: 77–90, http://dx.doi.org/10.1099/00221287-60-1-77

Vedia I, Oscoz J, Rueda J, Miranda R, García-Roger EM, Baquero E, Gelder SR (2014) An alien ectosymbiotic branchiobdellidan (Annelida: Clitellata) adopting exotic crayfish: a biological co-invasion with unpredictable consequences. *Inland Waters* 5: 89–92, http://dx.doi.org/10.5268/IW-5.1.799

Vogt G (1999) Diseases of European freshwater crayfish, with particular emphasis on interspecific transmission of pathogens. In: Gherardi F, Holdich DM (eds), Crayfish in Europe as alien species. How to make the best of a bad situation? Balkema, Rotterdam, pp 87–103

Wilcove DS, Rothstein D, Dubow J, Phillips A, Losos E (1998) Quantifying threats to imperilled species in the United States. *BioScience* 48: 607–615, http://dx.doi.org/10.2307/1313420

Williams BW, Gelder SR, Proctor HC, Coltman DW (2013) Molecular phylogeny of North American Branchiobdellida (Annelida: Clitellata). *Molecular Phylogenetics and Evolution* 66: 30–42, http://dx.doi.org/10.1016/j.ympev.2012.09.002

Yamaguchi H (1933) Description of a new branchiobdellid, *Cambarincola okadai* n. sp., parasitic on American crayfish transferred into a Japanese lake. *Proceedings of the Imperial Academy (Japan)* 9: 191–193